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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY PCT

(51) International Patent Classification 6 : A61L 33/00, 27/00, C08F 30/02, C07F 9/54	(11) International Publication Number: WO 95/20407
(21) International Application Number: PCT/GB95/00161	(81) Designated States: GB, JP, US, European patent AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE.
(22) International Filing Date: 27 January 1995 (27.01.95)	(43) International Publication Date: 3 August 1995 (03.08.95)
(30) Priority Data: PCT/GB94/00177 23 January 1994 (23.01.94) WO 9415926.5 4 August 1994 (04.08.94) GB	Published <i>With international search report.</i>
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(54) Title: NEW MATERIALS AND THEIR USE IN THE PREPARATION OF BIOMATERIAL SURFACES

(57) Abstract

New materials having improved biocompatibility have novel zwitterionic groups at the surface. The zwitterion is characterised by having at least one monovalent substituent at the atom carrying the cationic charge which is an aryl group. Preferably the atom carries at least two, and preferably three such aryl, usually phenyl, groups. The cationic group is preferably a triphenylphosphonium. Treatment of surfaces to provide pendant zwitterionic groups of the specified type have reduced platelet activation, whilst they do not appear to reduce platelet adhesion. The invention has utility in the field of blood contacting device, especially devices for implantation, such as vascular grafts.

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New materials and their use in the preparation of biocompatible surfaces

The present invention relates to new materials having improved biocompatibility and may be applied to or form a surface having improved biocompatibility. The materials have novel zwitterionic groups which are found to be less thrombogenic than existing materials, though having relatively high levels of platelet adhesion.

Materials used in medical devices where cell growth on the surface is desired, for example in cardiovascular stents and vascular grafts ideally must allow for the ready attachment of endothelial cells. As the mechanism of cell attachment and platelet adhesion are similar, it would be ideal if such a material did not activate platelets. Thus platelet adhesion without platelet activation would be a suitable combination of properties to ensure that thrombus formation does not occur, but endothelial cell growth is supported. With such a combination of properties, new devices which are currently impractical, such as small diameter vascular grafts, will become feasible.

It is known to reduce platelet activation by mimicking the surface of a red blood cell by coating a surface with phosphoryl choline zwitterionic groups. Various ways of introducing such groups at the surfaces of substrates are described in our earlier patent publications EP-A-0032622, EP-A-0157469, WO-A-9207858, WO-A-9113639, WO-A-9301221 and WO-A-9315775 and WO-A-9207885. In WO-A-9416748 (unpublished at the priority date of the present invention) various types of zwitterionic groups other than phosphoryl choline groups are described. For instance the trimethylammonium cation is replaced by various groups, including pyridinium groups. In other types of compound described in that document, the chain joining a phosphate ester and a quaternary nitrogen atom is increased in length or is an arylene group including heteroarylene. Further zwitterionic groups are also described in our earlier patent publication WO-A-9416749 (which was not published at

the priority date of the present invention). One exemplified compound disclosed in WO-A-9416748 has a triphenylphosphonium group as the cation of the zwitterion (the priority date for that specific compound is the same as in the present application). The present inventors have discovered that the triphenylphosphonium derivative appears to provide reduced platelet activation, but without reducing platelet adhesion. This is thought to be a result of the cation having at least one aryl group as a monovalent substituent and the present invention is based on that discovery.

In the present invention there is provided a surface having pendant zwitterionic groups in which the atom carrying the cationic charge has at least one monofunctional (or monovalent) (that is one of the substituents at that atom other than the substituent which joins the atoms to the anionic group and/or the surface) substituent which is an aryl, heteroaryl or aralkyl group.

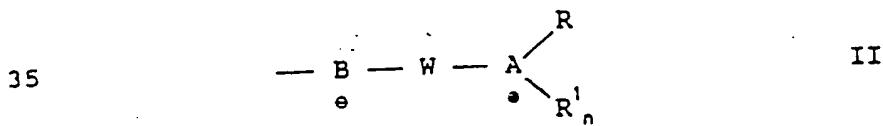
In the invention the pendant zwitterionic groups can be represented by the formula (I)



in which one of Y and Z is a cationic moiety having the defined aryl substituent, and the other of Y and Z is an anionic moiety and in which W is an alkylene (including cycloalkylene and cycloalkyl alkylene), arylene, heteroarylene and alkarylene, in which any alkylene chain, including cycloalkylene, may be interrupted by one or more oxygen atoms, and any of which groups may be unsubstituted or substituted by nonionic non-interfering substituents.

Preferably the cationic moiety is positioned further from the surface than the anionic moiety, that is Z represents the cationic moiety.

Preferably the zwitterionic group has the formula II:



in which B is a divalent anionic moiety,

W is as defined above,

A is a nitrogen atom, a phosphorus atom or a sulphur atom,

n is 1 when A is sulphur and

5 n is 2 when A is nitrogen or phosphorus,

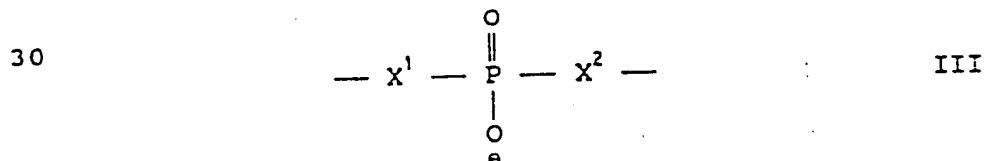
R is an aryl, heteroaryl or alkaryl group preferably aryl, usually phenyl,

and the or each R' is independently selected from aryl, heteroaryl, aralkyl and alkyl groups and hydrogen atoms. Preferably each R' is other than a hydrogen atom more preferably each is aryl, usually phenyl.

10 Preferably the group W separates the anionic and cationic moieties by a chain including at least 2 carbon atoms, preferably at least 5 carbon atoms and up to 20, more preferably up to 10 carbon atoms. The group W therefore preferably includes an alkylene group comprising at least 6, for instance at least 7, carbon atoms separating the ionic moieties. The group W is, preferably, alkylene of 5 or more, preferably at least 6, for instance 15 at least 7 carbon atoms optionally containing one or more ethylenically unsaturated double or triple bonds or oxygen atoms, arylene, alkarylene, aralkylene, alkylene aralkylene, cycloalkylene, alkylene cycloalkylene, most cycloalkylalkylene or alkylene cycloalkylene, most 20 preferably alkylene, optionally containing one or more fluorine substituents and/or one or more functional groups.

25 Preferably the group B in the general formula II is

III:

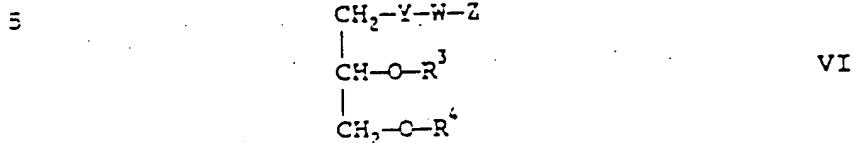


35 in which x¹ and x² are independently selected from a bond, -O-, -NH- and -S-. The group B is thus a phosphate ester group or a phosphate ester group in which one or both the oxygen atoms is replaced. Preferably the group B is a

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phosphate ester group, that is a group in which both X¹ and X² are oxygen atoms.

The group -YWZ may be part of a conjugated di-yne of the general formula VI:



10 in which at least one of R³ and R' is an acyl residue of a fatty acid having a conjugated di-yne group. Preferably both R³ and R' are such groups and are preferably the same. Where only one of R³ and R' is comprises a conjugated di-yne, the other is preferably a C₁₋₂₄, preferably C₈₋₂₀ alkyl group. The groups R³ and R' are suitably represented by the groups B₂ and B₃, respectively, specified in our earlier patent publication EP-A-0,032,622, the content of which is incorporated herein by reference. In particular the novel surface of the present invention may be provided by coating 15 a surface with compounds of the formula VI and, preferably, subsequently polymerising the compound in situ to form a crosslinked polymer containing repeat units analogous to those specified in EP-A-0,032,622.

20

Alternatively the group -YWZ may be part of a glyceride having the formula VI in which R³ and R' each represent C₈₋₂₄ alkyl or alkanoyl groups, in which the alkyl groups are usually straight chain alkyl. Such compounds may be coated onto hydrophobic substrate surfaces and retained by physisorption. Such methods are described in 25 our copending application, no. WO-A-92/06719, the content of which is incorporated herein by reference.

30 Alternatively the zwitterionic group at the surface may be provided by coating and reacting the surface with a compound of the formula IV:

35 $\text{R}^2 - \text{Y} - \text{W} - \text{Z}$ IV
 in which R² represents a group which reacts with or adsorbs onto the surface or reacts with a substrate polymer which can be coated onto the surface or which can polymerise or is a polymer residue. The substrate

generally comprises a polymer material, so that a reactive group R² includes a group which reacts with moieties on the substrate surface. The group at the polymer surface which reacts with the compound of the formula IV is generally a hydroxyl group, an amine group or a carboxylic acid group (-COOH). Where the polymer substrate has no such groups, it may be activated in an initial step, by known etching or derivatising techniques such as grafting, which introduce hydroxyl, carboxylic or primary or secondary amino groups on the surface (see for example "Chemical Reactions of Polymers", Ed EM Fettes (1964), Interscience Publishers, London). Suitable reactive groups as R² are described in our earlier patent publications nos. WO-A-91/06020, WO-A-91/13639, WO-A-92/07858, EP-A-0,157,469 and WO-A-93/05081, the contents of which are herein incorporated by reference. Particularly preferred reactive groups are activated amine groups such as are described in WO-A-9207858 and moieties which have the leaving groups discussed in WO-A-9113639.

The zwitterionic group may, as an alternative to being attached to the surface using a relatively small, low molecular weight reagent such as such classes of compound of the formula IV, be applied to a substrate as a coating material including a polymeric compound having pendant zwitterionic groups. A polymer having zwitterionic pendant groups may, for instance, comprise residues of a polymerisable compound containing a zwitterionic group YWZ. The polymer may be a homopolymer or copolymer of a polymerisable compound containing the zwitterionic group. Alternatively a polymeric compound containing such groups may be a polymer onto which the zwitterionic groups YWZ have been reacted, for instance using reactive groups of the type specified for R² above.

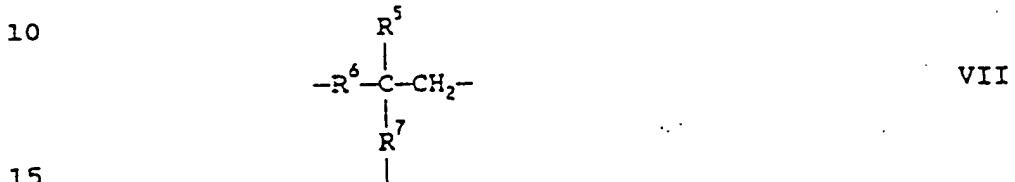
Such polymers may, for example, be condensation polymers such as polyesters, polyurethanes or polymers of ethylenically unsaturated compounds, such as polyolefins, poly(alk)acrylates, for examples polyacrylates or polymethacrylates, polystyrenes or polyvinyl polymers.

Specific examples of polymers containing zwitterionic groups used in the present invention are polyurethanes, for instance as described in our earlier patent publication no. WO-A-86/02933, polyesters such as described in our earlier 5 patent publication WO-A-88/00956 or, preferably, acrylate polymers such as described in WO-A-93/01221, the content of which are all incorporated herein by reference. Coating of substrates with grafted polymers is described in our earlier application WO-A-93/15775, which describes also 10 reaction of zwitterionic groups onto preformed polymers, especially naturally derived polymers or derivatives thereof, such as cellulosic polymers, and the content of that specification is incorporated herein by reference. Other polymers onto which zwitterionic groups have been 15 reacted by the free radical graft polymerisation of an ethylenically unsaturated compound containing zwitterionic groups, is described in our earlier application no. WO-A-93/05081, the content of which is incorporated herein by reference.

As an alternative to providing the pendant zwitterionic groups at the surface by coating a preformed 20 substrate, as described above, the zwitterionic groups may be present in the bulk of the material forming the substrate. The zwitterionic group may be present as a moiety of a lipid-like plasticiser molecule, for instance 25 analogous to the products described in our earlier application no. WO-A-87/02684, the content of which is incorporated herein by reference. Alternatively the zwitterionic group may be a pendant group of a polymer. 30 The polymer may be a polyurethane or a polyester, of the same type, though solid, as those described above as suitable for providing coatings. Preferably the polymer is formed from ethylenically unsaturated polymerisable compounds, such as polyolefins or, preferably, 35 poly(alk)acrylates. Such polymers are described in our earlier patent publication no. WO-A-92/07885, the content of which is incorporated herein by reference. Such

polymers may, for instance, comprise the residues of one or more diluent copolymerisable comonomers. The polymers may, further, be crosslinked, by incorporation of polyfunctional ethylenically unsaturated monomers which become incorporated into the polymer chains upon free radical polymerisation.

The surface of the present invention may comprise pendant groups of the formula IV in which R^2 has the formula VII

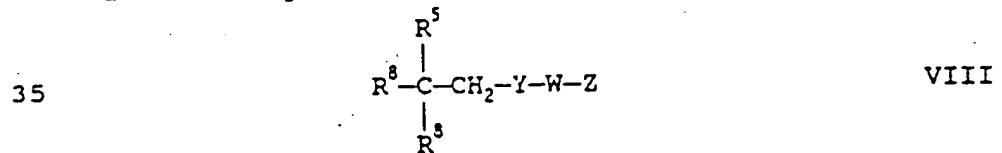


in which R⁵ is hydrogen, alkyl of 1-12 carbon atoms, preferably 1-4 carbon atoms, which is unsubstituted or substituted by a group capable of reacting with a hydroxyl, amino or carboxyl group, or with a biologically active compound, or R⁵ is a polymerisable, preferably ethylenically unsaturated group,

R⁶ and R⁷ are the same or different and are each selected from methylene or carbonyl groups, and through at least one of which the zwitterionic group is bound to the surface.

preferably each of R⁶ and R⁷ is methylene, and is preferably bound to an oxygen atom.

The present invention provides further novel compounds
of the formula IV in which R² is a moiety of the formula
VII, in which R⁶ and R⁷ are identical and are attached to
identical groups, especially groups of the formula VIII



in which the groups R³ are selected from any of the possible meanings of the group (R²-Y-) defined in our co-pending application no. WO-A-9416748, the content of which is incorporated herein by reference.

Preferably each R³ represents R⁹-E- where E is a valence bond or a linking group selected from -O-, -S-, -NH-, -OC(O)-, -C(O)-O-, -SC(O)-, -C(O)-S-, -NHC(O)-, -C(O)-NH-, -OC(O)O-, -SC(O)O-, -NHC(O)O-, -OC(O)S-, -SC(O)S-, -NHC(O)S-, -OC(O)NH-, -SC(O)NH- and -NHC(O)NH- and R⁹ is hydrogen, alkyl, alkenyl or alkynyl of up to 25 carbon atoms, optionally containing one or more etheric oxygen atoms and unsubstituted or substituted by one or more fluorine atoms and/or functional groups capable of reacting to provide covalent attachment to a surface and/or polymerisable groups.

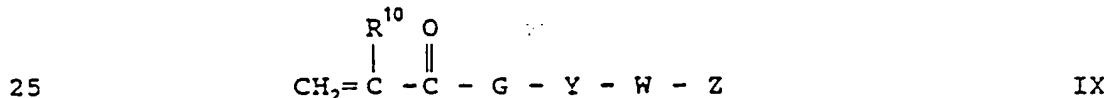
Most preferably E is -O- and R⁹ is a linear C₈₋₂₄ alkyl group or linear C₈₋₂₄ alkanoyl group.

The zwitterionic group may be a moiety on an analogue of a phospholipid, especially a fatty acid diester or diether of the formula VI, in which R³ and R⁴ represent saturated and unsaturated ester or ether residues derived from fatty acids or is a diester or diether of the formula VIII in which the groups R⁸ represent saturated or unsaturated ester or ether residues derived from fatty acids. Preferably the fatty acid residues are straight chain and contain from 12-20 carbon atoms. Such compounds may be used to coat hydrophobic substrates such as PVC, polyethylene or polypropylene by application of a solution in an organic solvent, followed by drying, as described in our co-pending application no. WO-A-92/06719, the content of which is incorporated herein by reference. Alternatively the phospholipid analogue may be incorporated by blending with a polymer compound, especially a thermoplastic polymer, when it behaves a simple external plasticiser, as described in our co-pending application no. WO-A-87/02684, the content of which is incorporated herein by reference.

Phospholipid analogues of the formula VI in which R³ and R⁴ represent saturated or unsaturated acyl or alkyl groups, especially C₁₂₋₂₀ straight chain alkyl are novel compounds.

The present invention provides also novel compounds of the formula IV, in which the group R² represents a moiety having a reactive group capable of reacting with hydroxyl, amine or carboxylic acid groups. Such compounds 5 are described above as reactants for derivatising a substrate surface.

The present invention provides also novel compounds of the formula IV in which the group R² is a polymerisable group. The group R² may be a difunctional moiety capable 10 of reacting in a condensation polymerisation process, for instance to form a polyurethane, polyester or polyamide. The functionalities may be selected from carboxylic acid, hydroxyl, amino or isocyanate. Alternatively the polymerisable moiety R² may comprise a free radical 15 polymerisable ethylenically unsaturated group, preferably a styrene, vinyl or (alk)acrylate moiety. Such compounds may be used to form addition homo or copolymers, as described above. Alternatively the ethylenically unsaturated moiety of R² may be graft polymerised onto a 20 substrate to form the surface having zwitterionic groups. A particularly preferred class of compounds has the general formula IX:



in which R¹⁰ is hydrogen or C₁₋₄ alkyl, preferably methyl, and G is -O-R¹² or -N(R¹¹)-R¹²- in which R¹¹ is hydrogen, C₁₋₄ alkyl or R¹²-Y-W-Z and R¹² is C₂₋₁₂ alkylene, preferably C₂₋₆ alkylene.

30 The present invention provides polymers, including homo and copolymers, of any such polymerisable compounds. An especially preferred class of polymer compounds comprises copolymers with copolymerisable monomer which provides a pendant group capable of providing stable 35 binding of the copolymer to a surface. The binding may be by covalent bond formed after coating of the preformed copolymer on to the surface, or by counter ionic bonding between oppositely charged groups on the polymer and on the

surface. Preferably the bonding is by hydrophobic interaction between a non polar, usually C₃₋₂₄ alkyl chain on the polymer and a hydrophobic surface. Especially preferred copolymers are of the zwitterionic (alk)acrylate monomer of the formula IX above and C₃₋₂₀ -alkyl (alk)acrylate monomer.

The present invention comprises especially novel compounds of any of the above classes in which the group W separates the anionic and cationic moieties by a chain including at least 2 carbon atoms, preferably at least 5 carbon atoms and up to 20, more preferably up to 10 carbon atoms. The group W therefore preferably includes an alkylene group comprising at least 6, for instance at least 7 carbon atoms separating the ionic moieties. The group W is, preferably, alkylene of 5 or more, preferably at least 6, for instance at least 7 carbon atoms optionally containing one or more ethylenically unsaturated double or triple bonds or oxygen atoms, arylene, alkarylene, aralkylene, alkylene aralkylene, cycloalkylene, alkylene cycloalkylene, cycloalkylalkylene or alkylene cycloalkylene, most preferably alkylene, optionally containing one or more fluorine substituents and/or one or more functional groups.

The invention provides further the use of compounds containing zwitterionic groups -YWZ in improving the biocompatibility and/or hydrophilicity (including lubricity) of a surface. It relates to a method of improving the biocompatibility and/or hydrophilicity of a surface by providing a surface having such pendant zwitterionic groups.

In the new use, a substrate is coated with a composition comprising a compound having a zwitterionic group -YWZ, so as to provide a surface having pendant zwitterionic groups YWZ. In the method the properties of the substrate are improved by reducing platelet activation, whilst platelet adhesion is substantially unaffected. Platelet adhesion may be increased or may be reduced to a

small extent, for instance by less than 50%, more preferably by less than 20% or less than 10%.

The tests for platelet adhesion and activation are described below. The platelet activation is preferably reduced by the method of the invention by at least 50%, preferably by at least 75%, by the provision of zwitterionic groups.

An alternative test for determining the effectiveness of the method of the present invention in reducing platelet activation is the determination of Factor XII activation. The test is described by E J Campbell, V O'Byrne, P W Stratford, I H Quirk, T A Vick, M C Wiles, and Y P Yianni in "Biocompatibles Studies using Methacryloylphosphoryl-choline Lauryl Methacrylate Copolymer", J Amer. Soc. Artificial Internal Organs, 1994, vol 40, No. 3, pM853-M857 and described below. The method of the present invention preferably reduces activated Factor XII in plasma by at least 50%, preferably at least 75% and sometimes as much as 90%, as compared to the amount of activated Factor XII produced in the presence of the untreated substrate. This test demonstrates the properties of the zwitterionic groups in reducing contact activation, a component of the intrinsic pathway of the coagulation cascade.

Substrates treated by the method of the present invention, as well as components having the specified surfaces may be used in a range of products where it is desired that cells are attached to the surface but thrombosis does not occur. The products are therefore preferably used in a blood or blood cell containing environments, especially on devices to be inserted, especially implanted, in the human or animal body, especially those implanted for an extended period of time, especially a week or more, for instance permanently. The invention is of particular value for vascular grafts and cardiovascular stents, especially relatively small diameter components of those types, or for wound-treatment products,

where it is desired for epithelial or other anchorage-dependent cells to grow.

The present invention is illustrated in the accompanying examples:

5 Test Methods

Platelet activation test

Blood was collected from a healthy adult volunteer using the double syringe method where the first 5ml of blood is discarded. The blood was collected into tri-sodium citrate (32g/l) in the proportion of 9 volumes to 10 1 volume citrate in plastic tubes. The samples were kept 1 volume citrate in plastic tubes. The samples were kept at room temperature on a spiral mixer until used.

Samples of polyethylene ribbon were treated with sample compound as described below and untreated 15 polyethylene ribbon were used as controls.

Half of the test samples were incubated with citrated blood (200 μ l) and the remainder were incubated with EDTA-treated blood on a phase shaker for 30 minutes before washing in PBS four times. Platelet adhesion was measured 20 in a manner similar to that described above for fibrinogen that is using enzyme immunoassay, using monoclonal antibodies to GPIb (cf Sheppeck RA *et al*, Blood (1991) 78:673 - 680). Platelet activation was measured in a manner similar to that described above for detection of 25 proteins by enzyme immunoassay using monoclonal antibodies against P-selectin (GMP140) to detect the presence of this platelet activation marker on the surface of biomaterials. In the presence of EDTA, which extracts calcium from inside 30 platelets, activation is inhibited, so that incubation with EDTA-treated blood acts as a non-specific control for activation, obviating the need for incubation in non-specific antibody.

Fibrinogen adsorption

The assay determines adsorption of human fibrinogen at 35 a surface. This protein is representative of protein which is typically adsorbed at a surface. The assay can be

readily modified to determine the adsorption of other proteins.

Polyethylene tubing was coated with a sample and human plasma (5 ml) was pumped through the tubing using a Watson-Marlow multi-head peristaltic pump (lowest setting). The tubing was then washed by pumping through phosphate buffered saline (PBS) (x2). A solution containing antibodies specific to human fibrinogen (5 ml) was then pumped through followed by a further wash of PBS (x2). A conjugate of horseradish peroxidase and a second antibody specific to the first fibrinogen-specific antibody (5 ml) was passed through followed by a further wash of PBS (x2). o-Phenylenediamine in phosphate-citrate buffer (5 ml), (0.8 mg/ml) was passed through and the absorption at 450 nm was read using a microplate reader.

Results are calculated as the percentage reduction in absorption at 450 nm compared to an untreated sample of polyethylene tubing. As a control for non-specific binding of antibody to the samples each sample was also incubated with non-specific antibody.

platelet adhesion by scanning electron microscopy

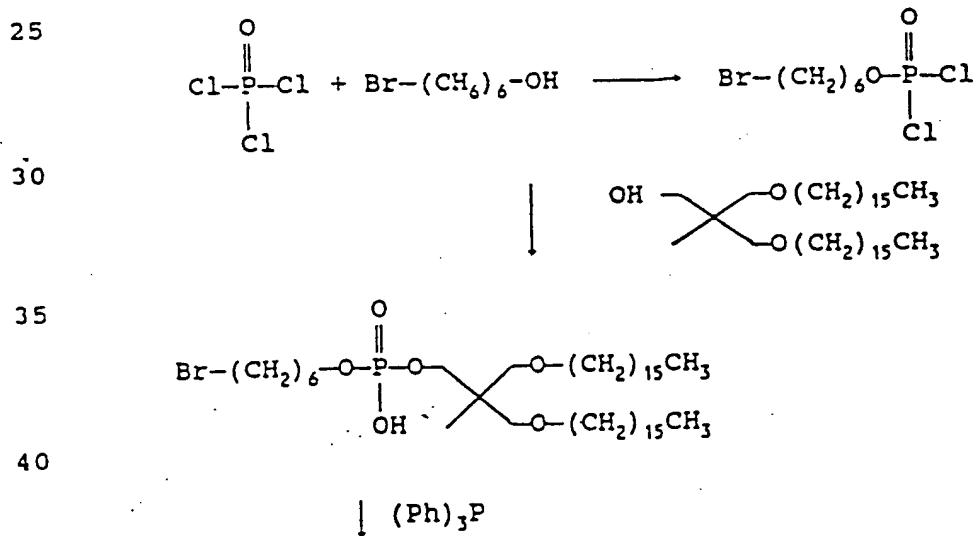
Samples were incubated in citrated blood, (9:1 32g/L), for 30 minutes at ambient temperature. Samples were then washed in phosphate buffered saline (PBS) and fixed with 2% glutaraldehyde in PBS for 15 minutes. Samples were then dehydrated using serial dilutions of alcohol (50-100%) and air dried. Samples were sputtered with gold and scanned in a S100 Scanning electron microscope. The micrographs show the presence and shape of platelets. From the characteristics shapes of activated and non-activated platelets, the extent of activation, if any, can be determined.

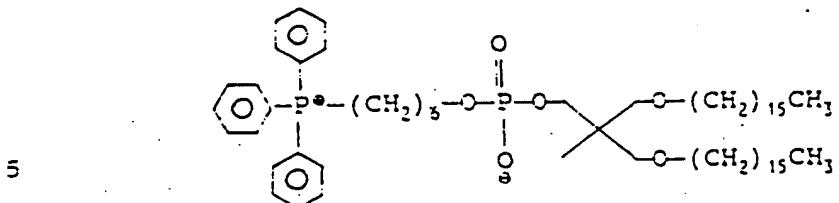
Factor XII Contact Activation Test

Contact activation of citrated fresh frozen plasma (FFP) by a compound under test onto a surface was determined using a commercially available activated Factor XII (FXIIa) kit produced by Shield Diagnostics (Dundee,

Scotland). 7 mm x 7 mm square samples of material were incubated in 250 μ l FFP, in a haemocompatible material coated microtiter plate, for 60 min. 100 μ l samples from each test well then were added to the precoated assay wells and incubated at ambient temperature for 60 min. Plasma samples were decanted and well washed four times using borate buffer. One hundred microliters Shield sheep anti-human FXIIa alkaline phosphatase labelled conjugate, in Tris buffer with a protein stabiliser, then was added to each test well. The plate was then incubated at ambient temperature for 60 mins and again washed 4 times with borate buffer and the plate blotted. One hundred microliters phenolphthalein monophosphate (PMP) solution, with Mg²⁺ enzyme cofactor, was added to each well and incubated for 10 min. Finally 100 μ l sodium hydroxide/ethylenediaminetetraacetic acid (EDTA) in carbonate buffer (pH > 10) was added to stop the reaction.

Example 1





6-Bromohexan-1-ol was added dropwise to phosphorusoxy chloride (equimolar) in dichloromethane over 30 minutes under nitrogen. The mixture was stirred at room temperature under nitrogen for 16 hours. The mixture was evaporated, dried under vacuum, then distilled under vacuum, to give the pure 6-bromohexyldichlorophosphate compound. Triethylamine and dichlorophosphate (equimolar) were added dropwise over 2 minutes under nitrogen to 1,3-dihexadecyloxy-2-(hydroxymethyl)-2-methyl propane ($0.3 \times$ molar amount of dichlorophosphate) in dry diethyl ether. The mixture was heated at 60°C under nitrogen for 16 hours before triethylamine (same molar amount as dichlorophosphate) and water were added and heating maintained for a further two hours. After cooling, the aqueous layer was removed and the residue evaporated to dryness and azeotroped with benzene ($\times 3$). The residue was dissolved in diethylether, filtered and evaporated to give a crude product, which was at least partially purified by silica gel chromatography using chloroform:methanol:25% ammonia (690:270:64). Triphenylphosphine was added to the intermediate thus prepared in dry chloroform. The mixture was heated at 60°C for 16 hours before it was cooled and the solvents evaporated under reduced pressure. The residue was purified by silica gel chromatography, eluting with chloroform:methanol (3:1) to chloroform:methanol:water (65:25:4). Fractions containing the desired compound were combined and evaporated under reduced pressure to give the desired final compound.

The compound was coated onto polyethylene strips from an ethanolic solution of 5 mg/ml. The coated strips were tested for fibrinogen adsorption. This test is generally used to indicate haemocompatibility. Normally, high fibrinogen binding indicates high platelet adhesion and

subsequent activation and thus poor haemocompatibility. The results of the test, that is of adsorbence at 450 nm was, for the uncoated polyethylene, 0.494 and, for the polyethylene coated with the triphenylphosphonium compound, 5 0.385. Both coated and uncoated polyethylene thus gave relatively high levels of fibrinogen adsorption.

The polyethylene coated with the triphenylphosphonium compound was assayed for platelet adhesion using an anti-GPIb antibody and platelet activation using an anti-P-selectin antibody. In these experiments, although both the 10 uncoated and coated polyethylene showed high levels of platelet adhesion, the coated material did not show the high level of platelet activation normally associated with this level of adhesion.

15 The results of the tests are as follows:

	<u>Uncoated</u> <u>Plt. adhesion</u>	<u>Uncoated</u> <u>Plt. activation</u>	<u>Coated</u> <u>Plt. adhesion</u>	<u>Coated</u> <u>Plt. activation</u>
Abs., 450 nm	0.479	0.550	0.456	0.125

20 The coated and uncoated samples were also subjected to the S.E.M. test for platelet adhesion. The micrographs of the coated sample reveal adhered platelets (as do the uncoated controls), though the platelets adhered to 25 coated samples show less activation than those on the uncoated controls.

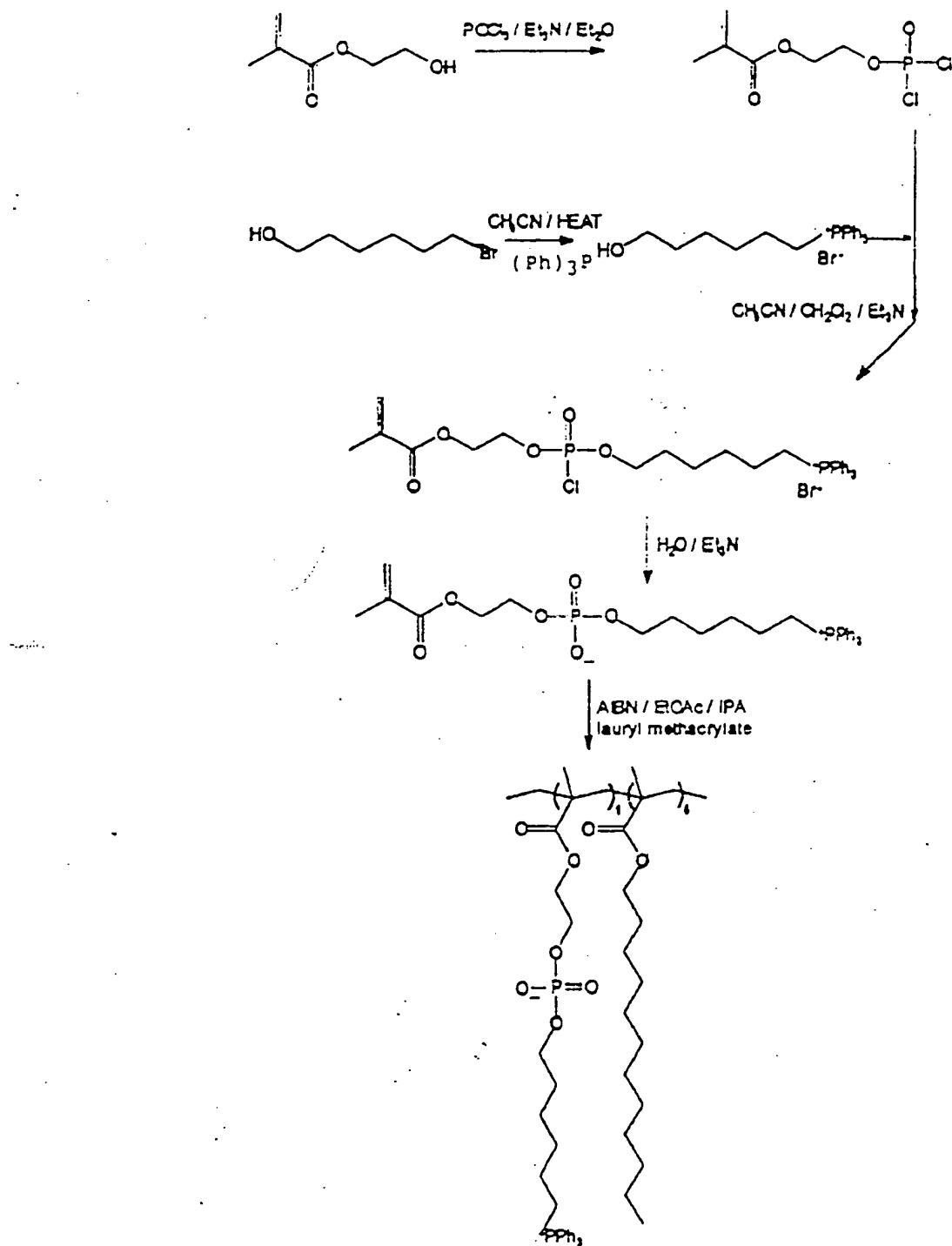
Thus the triphenylphosphonium compound provides a material which allows platelet adhesion without concomitant activation.

30 The amount of activated Factor XII (FXIIa) in plasma incubated with polyethylene or coated polyethylene was determined using the test described by Campbell *et al* (*op. cit.*). The level of FXIIa in plasma is indicative of the extent to which the contact activation of the coagulation 35 cascade has been activated. The results are as follows:

	<u>Uncoated</u>	<u>Coated</u>
ng/ml Factor XII above plasma level	3.804	0.359

The results of the Factor XIIa assay clearly demonstrate that the coated material is superior to the uncoated polyethylene in that it causes reduced activation of the coagulation cascade.

5 Example 2



To 6-bromohexan-1-ol (10 g) in dry acetonitrile (200 ml) was added triphenylphosphine (14.5g) and the mixture was heated in a sealed vessel at 70°C for 64 hours. The solvent was evaporated to dryness and the residue was dissolved in chloroform (150ml) and added dropwise to stirred diethylether (500ml). The solid was allowed to settle and the supernatant was decanted and the residue washed with diethylether (100ml) and dried under vacuum over phosphorus pentoxide. The solid was dissolved in dichloromethane (200ml) and subjected to Dean-Stark drying conditions at 70°C for six hours. The dried solution of hydroxyhexyltriphenylphosphonium bromide was kept until needed.

To a cooled (-10°C) solution of phosphorus oxychloride (6.20g) in dry diethylether (60ml) was added, dropwise, a mixture of 2-hydroxyethylmethacrylate (HEMA, 5.34g) and triethylamine (4.15g) in diethylether (40ml). The mixture was allowed to warm to room temperature and stirred for two hours. The solution was filtered and the filter bed washed with diethylether (30ml). The combined solution and washings were evaporated and the residue dissolved in acetonitrile (40ml) and added to the solution of hydroxyhexyltriphenylphosphonium bromide together with triethylamine (4.15g) and stirred at room temperature for 16 hours.

Water (10 ml) and triethylamine (4.15g) was added and stirring maintained for a further two hours. The solvent was evaporated, further water (30ml) was added and the solution was washed with a mixture of ethyl acetate and diethylether (1:1, 100ml). The aqueous layer was evaporated to dryness and azeotroped with benzene (x 3) to give a residue that was purified by silica gel chromatography (x 2) under a slight positive pressure, eluting with chloroform:methanol (9:1). Those fractions containing product were combined and evaporated to give a residue that was dissolved in chloroform (10ml) and washed

with water (10 ml). The organic layer was evaporated to give the monomer, 720mg.

¹H-nmr (200 MHz, CDCl₃), 1.40 - 1.80 (3H, s), 1.95 (3H, s), 3.65 (2H, m), 3.95 (2H, m), 4.10(2H, m), 4.30(2H, m)
5 5.50(1H, s), 6.10(1H, s), 7.60- 7.90(15H, m)

Monomer (320g) was dissolved in isopropyl alcohol (11.5ml) and lauryl methacrylate (2.53g) in ethyl acetate (3 ml) was added. The mixture was heated under a constant flow of nitrogen for 24 hours at 65°C after adding α -azo-
10 iso-butyronitrile (4mg in ethyl acetate) (1ml). The solvents were evaporated and the residue was redissolved in methanol (5ml) and dripped into hexane (100ml). The resulting solid was filtered off and the solvents were evaporated. The residue was titrated with hexane (x 3),
15 centrifuging the mixture between each wash. The resulting pellet was dissolved in a mixture of methanol and water (1:1, 30ml), which was then evaporated to remove most of the methanol to leave a white aqueous suspension, which was filtered and dried to give the desired product, 100mg.
20 ¹H-nmr (200 MHz, CDCl₃/CD₃OD), 0.8 - 2.0 (173H, m), 3.0-4.3 (20H, m), 7.6-8.0 (15H, m)

Gel permeation chromatography indicated an average molecular weight of 580000 when compared to polymethyl methacrylate standards.

25 The polymer was coated onto polyethylene strips from a 3mg/ml ethanolic solution. The coated strips were tested for fibrinogen adsorption. The results of the test, that is of absorbence at 450 nm was, for the uncoated polyethylene 0.898 and, for the polyethylene coated with 30 triphenylphosphonium compound, 0.600. Both coated and uncoated polyethylene thus gave relatively high levels of fibrinogen adsorption.

The polyethylene coated with the triphenylphosphonium polymer was assayed for platelet adhesion using an anti-GPIb antibody and platelet activation using an anti-P-selectin antibody. In these experiments, although both the coated and uncoated polyethylene showed high levels of

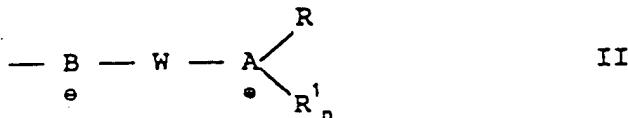
platelet adhesion, the coated material did not show the high level of platelet activation normally associated with this level of adhesion. Thus the triphenylphosphonium polymer provides a material which allows platelet adhesion without concomitant activation. The results of the tests are as follows:

		<u>Uncoated</u> <u>Plt. adhesion</u>	<u>Uncoated</u> <u>Plt. activation</u>	<u>Coated</u> <u>Plt. adhesion</u>	<u>Coated</u> <u>Plt. activation</u>
10	Abs., 450 nm	0.479	0.550	0.456	0.125

CLAIMS

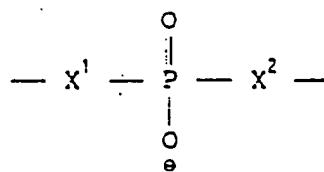
1. A surface having pendant zwitterionic groups in which the atoms carrying the cationic charge has at least one monofunctional substituent which is an aryl, heteroaryl or 5 aralkyl group.
2. A surface according to claim 1 in which the zwitterionic groups have the formula (I) -YWZ, in which one of Y and Z is a cationic moiety having the defined aryl substituent, and the other of Y and Z is an anionic moiety 10 and in which W is an alkylene (including cycloalkylene and cycloalkyl alkylene), arylene, heteroarylene or alkarylene group, in which any alkylene chain, including cycloalkylene, may be interrupted by one or more oxygen atoms, and any of which groups may be unsubstituted or 15 substituted by nonionic non-interfering substituents.
3. A substrate according to claim 2 in which Z is the cationic moiety.
4. A surface according to claim 2 or 3 in which the zwitterionic group has the formula II:

20



- in which B is a divalent anionic moiety,
W is as defined in claim 2,
25 A is a nitrogen atom, a phosphorus atom or a sulphur atom,
n is 1 when A is sulphur and
n is 2 when A is nitrogen or phosphorus,
R is an aryl, heteroaryl or alkaryl group, preferably 30 aryl, usually phenyl,
and the or each R' is independently selected from aryl, heteroaryl, aralkyl and alkyl groups and hydrogen atoms.
5. A surface according to claim 1 in which each R' is 35 aryl, preferably phenyl.
6. A surface according to claim 4 or claim 5 in which the group B is

5



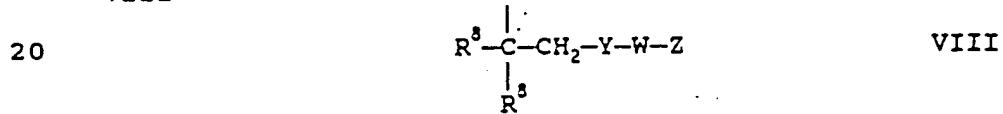
III

in which X^1 and X^2 are independently selected from a bond, $-\text{O}-$, $-\text{NH}-$ and $-\text{S}-$.

7. A surface according to any of claims 2 to 6 in which
10 W is an alkylene group comprising at least 6 carbon atoms separating the anionic and cationic moieties.

8. A surface according to any of claims 2 to 7 in which the group YWZ is part of a compound of the formula VI in which R³ and R⁴ represent the same or different C₁₋₂₄ alkyl
15 or alkanoyl groups.

9. A surface according to any of claim 2 to 7 in which the zwitterionic group is part of a compound of the formula
VIII

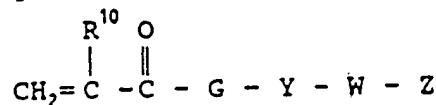


in which each R⁸ is the same and represents R⁹-E- where E
25 is a valence bond or a linking group selected from $-\text{O}-$, $-\text{S}-$, $-\text{NH}-$, $-\text{OC(O)}-$, $-\text{C(O)-O-}$, $-\text{SC(O)}-$, $-\text{C(O)-S-}$, $-\text{NHC(O)}-$, $-\text{C(O)-NH-}$, $-\text{OC(O)O-}$, $-\text{SC(O)O-}$, $-\text{NHC(O)O-}$, $-\text{OC(O)S-}$, $-\text{SC(O)S-}$, $-\text{NHC(O)S-}$, $-\text{OC(O)NH-}$, $-\text{SC(O)NH-}$ and $-\text{NHC(O)NH-}$ and R⁹ is hydrogen, alkyl, alkenyl or alkynyl of
30 up to 25 carbon atoms, optionally containing one or more etheric oxygen atoms and unsubstituted or substituted by one or more fluorine atoms and/or functional groups capable of reacting to provide covalent attachment to a surface and/or polymerisable groups.

35 10. A surface according to claim 9 in which E is $-\text{O}-$ and R⁹ is a linear C₈₋₂₄-alkyl or -alkanoyl group.

11. A surface according to any of claims 2 to 7 in which the zwitterionic group is part of a polymer produced by polymerising a monomer of the formula IX

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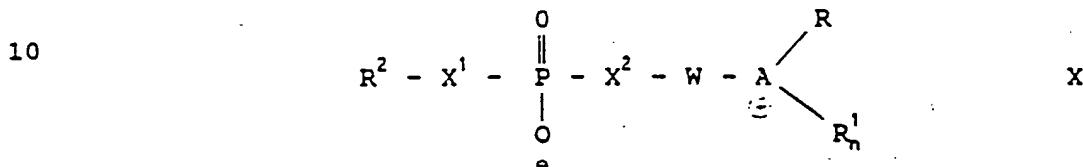


IX

in which R¹³ is hydrogen or C₁₋₆ alkyl, preferably methyl, and G is -O-R¹² or -N(R¹¹)-R¹²- and R¹¹ is hydrogen or C₁₋₆ alkyl or R¹²-Y-W-Z and R¹² is C₁₋₁₂ alkylene, preferably C₂₋₆ alkylene.

5 12. A surface according to claim 11 in which the polymer is a copolymer of the monomer of the formula IX and a copolymerisable C₈₋₂₄ -alkyl(alk) acrylate.

13. A compound of the formula X



15 in which B is a divalent anionic moiety,
W is as defined in claim 2,
A is a nitrogen atom, a phosphorus atom or a sulphur atom,

n is 1 when A is sulphur and

20 n is 2 when A is nitrogen or phosphorus,

R is an aryl, heteroaryl or alkaryl group, preferably aryl, usually phenyl,

and the or each R¹ is independently selected from aryl, heteroaryl, aralkyl and alkyl groups and hydrogen atoms,

25 X¹ and X² are independently selected from a bond, -O-, -NH- and -S- and

30 R² represents a group which can react with or adsorbs onto a surface or can react with a polymer or which can polymerise or is a polymer residue.

14. A compound according to claim 13 in which R² is a group



35 in which each R³ is the same and represents R⁹ -E- where E is a valence bond or a linking group selected from

- O-, -S-, -NH-, -OC(O)-, -C(O)-O-, -SC(O)-, -C(O)-S-,
 -NHC(O)-, -C(O)-NH-, -OC(O)O-, -SC(O)O-, -NHC(O)O-,
 -OC(O)S-, -SC(O)S-, -NHC(O)S-, -OC(O)NH-, -SC(O)NH- and -
 NHC(O)NH- and R' is hydrogen, alkyl, alkenyl or alkynyl of
 5 up to 25 carbon atoms, optionally containing one or more
 etheric oxygen atoms and unsubstituted or substituted by
 one or more fluorine atoms and/or functional groups capable
 of reacting to provide covalent attachment to a surface
 and/or polymerisable groups.
- 10 15. A compound according to claim 14 in which E is -O- and
 R' is a linear C₃₋₂₄ -alkyl or -alkanoyl group.
16. A monomer of the formula IX
- $$\text{CH}_2=\text{C}(\overset{\text{R}^{10}}{\underset{|}{\text{O}}})\text{G}-\text{Y}-\text{W}-\text{Z}$$
- 15 in which R¹⁰ is hydrogen or C₁₋₄ alkyl, preferably methyl,
 and G is -O-R¹² or -N(R¹¹)-R¹²-, in which R¹¹ is hydrogen, C₁₋₄
 alkyl or R¹²-Y-W-Z and R¹² is C₁₋₁₂ alkylene, preferably C₂₋₆
 alkylene.
- 20 17. A polymer formed by polymerising a monomer according
 to claim 16.
18. A polymer according to claim 17 in which the monomer
 is copolymerised with a copolymerisable C₃₋₂₄ -alkyl
 (alk)acrylate, preferably a C₁₂₋₁₈ linear alkyl methacrylate,
 25 comonomer.
19. A process in which a substrate surface is coated with
 a compound comprising a zwitterionic group as defined in
 any of claims 1 to 12.
20. A process according to claim 19 in which the compound
 30 is a polymer according to claim 17 or claim 18.
21. Use of a zwitterionic group as defined in any of
 claims 1 to 12 to improve the biocompatibility,
 haemocompatibility and/or hydrophilicity of a substrate.
22. Use according to claim 21 in which the adhesion of
 35 platelets to the substrate is substantially unaffected by
 the zwitterionic groups.

25

23. A blood contacting device having a blood contacting surface which is a surface according to any of claims 1 to 12.
24. A device according to claim 23 which is an implantable prostheses.
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INTERNATIONAL SEARCH REPORT

Intern. App. No.
PCT/GB 95/00161

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61L33/00 A61L27/00 C08F30/02 C07F9/54

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61L C07F C08F

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO,A,91 13639 (BIOCOMPATIBLES LTD.) 19 September 1991 see the whole document ---	1-24
Y	WO,A,92 07858 (BIOCOMPATIBLES LTD.) 14 May 1992 see the whole document ---	1-24
Y	WO,A,92 21386 (BIOCOMPATIBLES LTD.) 10 December 1992 see the whole document ---	1-24
Y	EP,A,0 032 622 (CHAPMAN, DENNIS) 29 July 1981 see the whole document ---	1-24
		-/-

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

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Date of the actual completion of the international search

Date of mailing of the international search report

15 March 1995

29.03.95

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Beslier, L

INTERNATIONAL SEARCH REPORT

Intern. Application No.
PCT/GB 95/00161

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PHARMAZIE (PHARAT,00317144);79; VOL.34 (12); PP.845, KARL-MARX-UNIV.;SEKT. BIOWISS.; LEIPZIG; DDR-701; GER. DEM. REP. Kertscher P et al 'Trimethylammonium group substitution of phosphatidylcholine by other onium groups' see the whole document ---	13-15
P,Y	WO,A,94 16748 (BIOCOMPATIBLES LTD.) 4 August 1994 cited in the application ---	i-24
P,Y	WO,A,94 16749 (BIOCOMPATIBLES LTD.) January 1994 cited in the application see particularly example 7 -----	1-24

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Inten. Application No
PCT/GB 95/00161

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WO-A-9221386	10-12-92	EP-A- JP-T-	0641226 6507558	08-03-95 01-09-94
EP-A-0032622	29-07-81	JP-C- JP-B- JP-A- JP-C- JP-B- JP-A- JP-C- JP-B- JP-A- JP-C- JP-B- US-A-	1684880 3047892 63240938 1685718 3051429 63277064 1656304 3014838 56135492 1158013 1829269 5025883 4348329	31-07-92 22-07-91 06-10-88 11-08-92 06-08-91 15-11-88 13-04-92 27-02-91 22-10-81 21-06-89 15-03-94 14-04-93 07-09-82
WO-A-9416748	04-08-94	NONE		
WO-A-9416749	04-08-94	NONE		